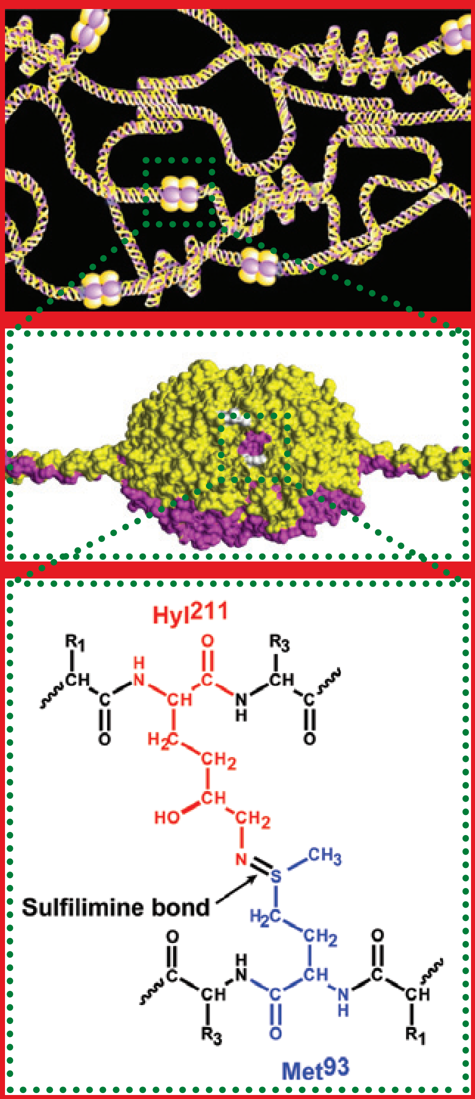

Researchers have recently discovered a new type of chemical bond in collagen IV proteins (model depicted in upper panel). Collagen IV proteins form a network that provides the structural integrity of the glomerular basement membrane. The collagen IV network is stabilized by crosslinks or bonds between different collagen IV proteins. It was known that a bond was established between two of the building blocks of proteins: a methionine amino acid (Met) and a modified lysine amino acid called hydroxylysine (Hyl). However, the specific nature of the bond remained elusive, despite decades of investigation. Recently, using cutting-edge instrumentation, the scientists discovered that these collagen IV components are linked together with a "sulfilimine bond" (bottom panels). This type of chemical bond had never before been identified in a biological specimen; scientists speculate that it may add strength to the collagen IV network. For more on this research advance and how it relates to an autoimmune kidney disease called Goodpasture's syndrome, please see the write-up later in the chapter.

Graphics provided by Dr. Billy G. Hudson from Vanacore R, Ham A-JL, Voehler M, Sanders CR, Conrads TP, Veenstra TD, Sharpless KB, Dawson PE, and Hudson BG: A sulfilimine bond identified in collagen IV. Science 325: 1230-1234, 2009. Reprinted with permission from AAAS.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. They afflict millions of Americans, including children and young adults. The NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The goal is to increase understanding of kidney, urologic, and hematologic diseases to enhance prevention and treatment strategies.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, even for a short period of time or due to gradual deterioration, can result in life-threatening complications. Whether kidney function is lost suddenly or slowly represents an important health challenge.

Chronic kidney disease has two main causes: high blood pressure and diabetes. Recent estimates put the number of Americans with chronic kidney disease at more than 23 million.[1] If unchecked, the recent increases in obesity and type 2 diabetes in the U.S.—especially among children and adolescents—have grave implications, as individuals are likely to face any secondary health consequences at an earlier age than people who develop these conditions as middle-aged adults.

Chronic kidney disease, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. At the close of 2007, more than 525,000 patients were receiving treatment for ESRD: nearly 370,000 were undergoing dialysis and almost 160,000 were living with a kidney transplant. Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease and ESRD. African Americans are four times more likely and American Indians are twice as likely to develop kidney failure as non-Hispanic whites. Hispanics have a significantly increased risk for kidney failure as well.[2]

The NIDDK supports a significant body of research aimed at understanding the biology underlying chronic kidney disease. The Institute's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease; the underlying mechanisms leading to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Also of interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome. The Institute's National Kidney Disease Education Program is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure. It represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research effort includes basic, clinical, and epidemiologic research on the genitourinary tract.

Benign prostatic hyperplasia (BPH) is an enlargement of the prostate—a gland below the bladder that surrounds the urethra—a common condition affecting about 50 percent of men in their 50s. When enlarged, the prostate can restrict urine flow from the bladder. Lower urinary tract symptoms (LUTS) are the

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[1] *Levey AS, et al: Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2009 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the U.S., National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2009.*

symptoms thought to be related to BPH; however LUTS are not exclusive to BPH or men.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating, chronic, and painful bladder disease. IC/PBS affects both men and women, but it is nine times more common in women. In men, prostatitis—chronic, painful inflammation of the prostate gland—accounts for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genitourinary system.

NIDDK-supported basic and clinical research is focused on elucidating the causes of IC/PBS, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. The Interstitial Cystitis Clinical Trials Group/Research Network conducts clinical studies in IC/PBS. NIDDK's Multidisciplinary Approach to the Study of Urologic Chronic Pelvic Pain (MAPP) Syndromes Research Network supports studies designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. The goals and approaches of the MAPP Research Network reflect the most current thinking on IC/PBS pathology and involve significant new advancements in how IC/PBS is studied. All efforts are designed to provide insights that can be translated to improve the clinical care of IC/PBS patients.

A conservative estimate is that approximately 13 million Americans, most of them women, suffer from urinary incontinence.[3,4] Many suffer in silence due to embarrassment and lack of knowledge about options available. The introduction of new surgical procedures has advanced the treatment of urinary incontinence dramatically in the last decade. The NIDDK's Urinary Incontinence Treatment Network recently completed the Trial of Mid-Urethral Slings (TOMUS) study comparing two minimally invasive surgeries for the treatment of stress urinary incontinence and results are expected in 2010.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. The Institute is also keenly interested in the basic biology of stem cells, including adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

DELVING INTO COLLAGEN NETWORKS

A New Chemical Bond in Collagen IV Networks: Researchers have discovered a new kind of chemical bond in biological tissue, a fundamental discovery in structural biology that may provide insights into several human diseases. The bond connects two of the subunits that make up collagen IV, a protein that is an important component of the extracellular matrix. Collagen provides structural support to tissues, serves as a scaffold upon which other complexes are assembled, and mediates cellular signaling. Scientists have long known that collagen subunits are linked to one another through intermolecular bonds that lend strength and structural integrity to the matrix; however, the precise nature of these bonds had eluded them. Using advanced techniques to study protein structure, the researchers discovered a novel chemical bond between a sulfur atom of the amino acid methionine on one subunit and a nitrogen atom of a modified form of the amino acid lysine on another. This is the first time that a "sulfilimine" bond—a direct bond between sulfur and nitrogen atoms—has been found in a native biomolecule.

[3] *Nygaard I, et al: Urinary Incontinence in Women in Urological Diseases in America (pp. 157-191). NIDDK, NIH Publication Number 07-5512, 2007.*

[4] *Stothers L, et al: Urinary Incontinence in Men in Urological Diseases in America (pp. 193-221). NIDDK, NIH Publication Number 07-5512, 2007.*

Collagen IV networks have been implicated in a number of human diseases. Two collagen subunits are defective in the inherited kidney disorder Alport syndrome, a condition in which waste filtering by the kidney is impaired due to disruption of the extracellular matrix. Furthermore, one collagen chain is involved in the rare, autoimmune kidney disease, Goodpasture's syndrome. In this disease, the misguided antibody attack on the kidney's extracellular matrix is thought to be due to exposure of a usually hidden part of the collagen molecule, which may be made accessible as a consequence of the absence or breakage of the sulfilimine bond. Discovery of this novel chemical bond therefore represents not only an important advance in our knowledge of collagen structure, but also may identify causes of disease and possible treatment approaches.

Vanacore R, Ham AJ, Voehler M, Sanders CR, Conrads TP, Veenstra TD, Sharpless KB, Dawson PE, Hudson BG: A sulfilimine bond identified in collagen IV. Science 325: 1230-1234, 2009.

GENETICS OF KIDNEY DISEASE

Common Genetic Variants as Contributors to Risk of Chronic Kidney Disease: Researchers have identified variations at five distinct genetic regions (loci) that are associated with either diminished kidney function or chronic kidney disease. The scientists performed genome-wide association scans on nearly 20,000 biological samples that had been collected from volunteers during previous trials, and then replicated these results with a separate set of over 21,000 additional specimens. They found that variations in the *UMOD* genetic locus were associated with chronic kidney disease; that variations at the *UMOD*, *SHROOM3*, and *GATM-SPATA5L1* loci were associated with diminished kidney filtration rates as determined by mathematically extrapolating from serum creatinine levels; and that variations at the *CST* and *STC1* loci were associated with diminished kidney filtration rates extrapolated from serum cystatin C levels.

Several of these genes are likely to play a direct role in kidney function. The *UMOD* gene encodes the most abundant protein found in the urine of healthy people, although its biological function is unclear. The *SHROOM3* gene product is present in human kidneys and is thought to play a role in the regulation of cell shape. And the *SCT1* gene product may help regulate calcium and phosphate balance. The discovery and validation of several common variants at previously unidentified genetic regions that are associated with increased risk of diminished kidney function and chronic kidney disease have important implications for both researchers and clinicians. Further studies to understand the role of these proteins in kidney disease—including discovering the function of the protein encoded by the *UMOD* gene—may lead to new approaches to prevent and treat chronic kidney disease.

Köttgen A, Glazer NL, Dehghan A, Hwang SJ, Katz R, Li M, Yang Q, Gudnason V, Launer LJ, Harris TB, Smith AV, Arking DE, Astor BC, Boerwinkle E, Ehret GB, Ruczinski I, Scharpf RB, Chen Y-DI, de Boer IH, Haritunians T, Lumley T, Sarnak M, Siscovick D, Benjamin EJ, Levy D, Upadhyay A, Aulchenko YS, Hofman A, Rivadeneira F, Uitterlinden AG, van Duijn CM, Chasman DI, Paré G, Ridker PM, Kao WH, Witteman JC, Coresh J, Shlipak MG, and Fox CS: Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet 41: 712-717, 2009.

KIDNEY FIBROSIS RESEARCH

Identification of the Cellular Source of Scar-Producing Collagen in a Model of Kidney Fibrosis: Scientists have recently pinpointed a type of cell in the kidney that appears to play a major role in tissue scarring that is seen in some forms of kidney disease. Fibrosis is the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to scarring within an organ. It is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis can impair the removal of toxins and excess fluid from the blood, cause irreversible kidney damage and, in extreme cases, lead to kidney failure. Therefore, the causes of fibrosis are of great interest to researchers and physicians.

To study the molecular and cellular biology of fibrosis in the kidney, researchers used mice in which cells that produce a particular form of collagen were "tagged" with a fluorescent protein, allowing them to be clearly identified. They then used surgical obstruction of one ureter—the conduit through which urine collected by the kidney flows to the bladder—to induce kidney inflammation and subsequent fibrosis. Following ureteral obstruction, cells known as myofibroblasts were found to be the major source of collagen in the fibrotic kidneys. Interestingly, the vast majority of myofibroblasts in the kidney were derived from pericytes, cells that have the potential to differentiate into other cell types and are often found associated with the walls of small blood vessels, including those that pass through the kidney. The identification of pericytes, which are not derived from the kidney's nephrons but rather from its associated vasculature, as the source of collage-producing myofibroblasts will likely focus renewed attention on the role of the circulatory system in triggering and/or mediating kidney fibrosis. Understanding the origin of the scar-producing cells is a key step in elucidating the mechanisms through which fibrosis develops in response to kidney injury or inflammation, and may identify new targets for future therapeutic interventions.

Lin S-L, Kisseleva T, Brenner DA, and Duffield JS: Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol 173: 1617-1627, 2008.

AUTOIMMUNE KIDNEY DISEASE

Potential Immune System Target Antigen in Autoimmune Kidney Disease: Researchers have identified a protein that may play a key role in the development of an autoimmune form of kidney disease known as "idiopathic membranous nephropathy." This type of kidney disease is a common cause of nephrotic syndrome in adults. It is an autoimmune disease, meaning that the body's immune system incorrectly mounts an attack against a normally occurring protein in the body. The disease is characterized by protein in the urine, lowered protein levels in the blood, elevated cholesterol, and swelling of the face, hands, and feet. The identification of the protein that induces this immune response (termed an "antigen") will open new avenues of exploration in idiopathic membranous nephropathy.

To identify potential target antigens, scientists collected blood samples from patients with idiopathic membranous nephropathy, and mixed them with proteins that were obtained from kidney tissue. In 70 percent of the tested blood samples, self-reactive antibodies (autoantibodies) identified a single kidney protein that was ultimately determined to be the M-type phospholipase A_2 receptor or PLA_2R. This protein is expressed by cells in glomeruli, tiny filtering units in the kidney that are injured in this syndrome. The subtype of antibody that reacted with PLA_2R in the assay is the same kind that is found in immune deposits within the glomeruli in patients with this disease. Antibodies isolated from glomeruli of patients with idiopathic membranous nephropathy react with PLA_2R, whereas antibodies isolated from the glomeruli of patients with nephropathy arising from other causes do not. Furthermore, there is evidence to suggest that, in patients with clinically significant disease activity, autoantibodies against PLA_2R can be readily detected in the blood. In contrast, in patients in whom the disease is in remission, levels of these antibodies decline or disappear.

Fifty years ago, researchers studying a rat model of idiopathic membranous nephropathy identified a kidney protein that appeared to be an immunological target for autoantibodies; however, progress stalled when this protein was found to be absent in human kidneys. These new findings will also have important implications for patient care. For example, they may permit the noninvasive diagnosis of membranous nephropathy, as well as provide an easier way to follow the disease in response to treatment. Better understanding of the potential triggers of autoantibody production in patients with a susceptibility to idiopathic membranous nephropathy may also uncover possible new targets for preventing or treating this disease.

Beck LH Jr, Bonegio RGB, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, and Salant DJ: M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med 361: 11-21, 2009.

New Insight into the Immunology of Goodpasture's Syndrome: Scientists have recently reported new findings on the immunological events relevant to a debilitating autoimmune disease known as Goodpasture's syndrome (GPS). This disease is a rare condition marked by kidney damage—sometimes leading to kidney failure—and bleeding in the lungs. Its precise underlying cause is unknown but, as with all autoimmune diseases, it arises when the cells of the immune system wrongly recognize "self" molecules as foreign and initiate an attack on them. Under normal circumstances, developing immune cells that react with proteins that occur naturally in the body are selectively deleted. For example, the B cells of the immune system produce antibodies to attack invaders such as infectious agents. However, if a particular B cell's antibodies happen to react to one of the body's own proteins, that B cell is either eliminated, altered to produce different antibodies, or otherwise neutralized. This process is known as "tolerance." It has not been clear why people with Goodpasture's syndrome, or other autoimmune diseases, have self-reactive antibodies. One theory is that, in people with autoimmune diseases such as GPS, potentially self-reactive immune cells are not deleted as they should be. Another theory is that non-self-reactive immune cells may undergo chromosomal rearrangement, rendering them self-reactive.

To explore how self-reactive antibodies in Goodpasture's syndrome could escape tolerance-induced deletion, a group of researchers generated a mouse model to study based on knowledge of the target of these antibodies. In previous research, scientists had identified a region of the alpha3 chain of type IV collagen—a component of connective tissue present in the kidneys and the lungs—as the target of this immune attack. This target is normally hidden amid the other portions of collagen, and exists only in a few body tissues. Thus, it was speculated that this region of collagen may not be sufficiently available to developing B cells for the body to screen out any that produce reactive antibodies. In Goodpasture's syndrome, it was thought that perhaps this collagen target later becomes aberrantly available to the B cells, after the normal time at which tolerance occurs, resulting in an antibody reaction and tissue damage. In the current study, researchers engineered mice with B cells producing antibodies that recognize the same region of collagen as the immune cells in people with GPS. Surprisingly, the researchers found that the B cells producing self-reactive antibodies were selectively eliminated in the bone marrow, before they had a chance to circulate throughout the body. The researchers then investigated whether any of the B cells might have altered their antibody structures so they would no longer react against collagen. To assess this possibility, they compared antibodies in these mice and in mice lacking a gene, called *Rag*, which is required for this type of antibody alteration. They found that some of the formerly self-reactive B cells appeared to have modified their antibodies; mice deficient in the *Rag* gene did not have these altered antibodies.

These findings overturn previous speculation about the origin of self-reactive antibodies in Goodpasture's syndrome. The study suggests that, in most individuals, auto-reactive B cells are either eliminated or sufficiently modified in the bone marrow during maturation. The development of Goodpasture's syndrome may thus result from either the escape of a few collagen-reactive immune cells, perhaps accompanied by other disease triggers, or from changes to B cells following self-tolerization.

Zhang Y, Su SC, Hecox DB, Brady GF, Mackin KM, Clark AG, and Foster MH: Central tolerance regulates B cells reactive with Goodpasture antigen alpha3(IV)NC1 collagen. J Immunol 181: 6092-6100, 2008.

POTENTIAL CAUSE OF REDUCED KIDNEY FUNCTION AND KIDNEY STONE FORMATION

Genome-Wide Scan Identifies Genes Linked to Increased Risk of Gout: Researchers have recently reported the identification of two new genes and confirmed the previous finding of a third gene associated with increased levels of uric acid in the blood and increased risk of gout, a form of arthritis. Chronic gout is associated with increased risk of kidney stone formation and decreased kidney function. Among the known risk factors for gout, hyperuricemia is a condition in which there is an excess level of uric acid in the blood. Under circumstances that are not yet completely understood, the excess uric acid begins to crystallize, causing inflammation and pain in the joints. Previous studies have identified a type of variation in DNA sequence, called single nucleotide polymorphisms (SNPs), in the *SLC2A9* gene that correlated with increased levels of uric acid and gout.

Researchers sought to identify additional genes that contribute to increased uric acid levels and gout. They conducted genome-wide association studies on samples from participants of several large studies—7,699 participants in the well-characterized Framingham Heart Study and 4,148 from the Rotterdam Study—to first identify genetic regions associated with increased uric acid levels. They then confirmed those findings in samples from 14,867 participants in another study, the Atherosclerosis Risk in Communities study. Subsequent analysis determined whether SNPs associated with increased uric acid levels are linked to the development of gout. Two new genes, *ABCG2* and *SLC17A3*, and the previously identified gene *SCL2A9*, were shown to contain SNPs that were associated with both increased uric acid levels and gout. All three of these genes code for proteins suspected of transporting uric acid and potentially other small molecules across the kidney cell membrane. Interestingly, the SNPs for *SCL2A9* and *ABCG2* were associated with increased uric acid levels in both white and African American participants, whereas the variant for *SLC17A3* correlated only with increased uric acid levels in whites.

The investigators then determined whether these gene variants were associated with the development of gout. In contrast to the SNP for *ABCG2*, which was associated with gout in both white and African American participants, the variants for *SCL2A9* and *SLC17A3* were associated with gout in white participants only.

This study identifies additional genes having a role in uric acid buildup and development of gout. It also reinforces the concept that gene variants may act differently on different genetic backgrounds. This knowledge may lead to new treatment strategies as well as the development of drugs with fewer side effects compared to those currently available.

Dehghan A, Köttgen A, Yang Q, Hwang S-J, Kao WHL, Rivadeneira F, Boerwinkle E, Levy D, Hofman A, Astor BC, Benjamin EJ, van Duijn CM, Witteman JC, Coresh J, and Fox CS: Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet 372: 1953-1961, 2008.

DEVELOPING NEW TREATMENTS FOR KIDNEY DISEASE

Combination of Aspirin and an Anti-Clotting Drug Reduces Risk of Dialysis Access Failure: For the first time, a combination of aspirin and the anti-platelet drug dipyridamole has been shown to significantly reduce blockages and extend the useful life of new artery-vein (AV) access grafts used for hemodialysis, in a study conducted by the NIDDK-supported Dialysis Access Consortium (DAC). Patients undergoing dialysis for kidney failure sometimes have a synthetic tube, or graft, implanted under the skin of the arm. The graft becomes an artificial vein that can be used repeatedly for needle placement and blood access during hemodialysis. When these access grafts fail, it is most often due to narrowing of blood vessels at the graft site (termed "stenosis") and subsequent clotting, both of which can diminish or block the flow of blood. A blocked graft cannot be used for dialysis and is a major cause of deteriorating health in dialysis patients.

The DAC trial enrolled a total of 649 participants with new AV grafts who were randomly assigned to receive either aspirin and dipyridamole or a placebo. Both aspirin and dipyridamole inhibit platelet aggregation, which can result in the formation of blood clots. The trial found that the combination drug treatment decreased the rate of loss of the useful life of a graft before it becomes blocked the first time by 18 percent, and the rate of developing significant stenosis by 28 percent, compared to placebo, modestly but significantly prolonging the viability of AV grafts. However, the overall rates of graft failure, complications such as bleeding, and death were similar in both groups. This finding validates an approach that may help to maintain AV grafts so that patients can continue to receive life-sustaining dialysis. It is also a step forward in the development of therapies that improve the quality of life for dialysis patients. In order to improve long-term graft viability, future studies may address the underlying causes of vascular stenosis at the graft site.

Dixon BS, Beck GJ, Vazquez MA, Greenberg A, Delmez JA, Allon M, Dember LM, Himmelfarb J, Gassman JJ, Greene T, Radeva MK, Davidson IJ, Ikizler TA, Braden GL, Fenves AZ,

Kaufman JS, Cotton JR Jr, Martin KJ, McNeil JW, Rahman A, Lawson JH, Whiting JF, Hu B, Meyers CM, Kusek JW, Feldman HI, for the DAC Study Group: Effect of dipyridamole plus aspirin on hemodialysis graft patency. N Engl J Med 360: 2191-2201, 2009.

Potential New Therapy for Patients with the Kidney Disease Nephrogenic Diabetes Insipidus: NIH scientists have recently developed the first viable animal model of the kidney disease nephrogenic diabetes insipidus (NDI) and have shown that drug treatment can greatly reduce the manifestations of the disease. The vast majority of patients with NDI have a mutation resulting in loss of function of the V2 vasopressin receptor (*V2R*) gene. Because the *V2R* gene is located on the X chromosome, this form of NDI is called X-linked NDI or XNDI and, as with other X-linked diseases, it is seen almost exclusively in males. In people with XNDI, the kidneys are unable to effectively reabsorb water during filtration, leading to the excretion of large volumes of dilute urine, which can result in electrolyte imbalance and dehydration. In severe cases, patients are at risk for kidney damage and failure, mental retardation, or—in undiagnosed infants and children—failure to thrive and death. Currently, there is no effective treatment for XNDI, and research has been hampered by the absence of good animal models in which to study the disease and to test potential therapies.

NIDDK intramural scientists, in collaboration with National Heart, Lung, and Blood Institute scientists, developed a mouse model in which the *V2R* gene could be selectively deleted in adult animals. Deletion of this gene resulted in the appearance of disease symptoms that very closely resembled human XNDI. The mice excreted large amounts of dilute urine, did not gain weight as fast as normal mice, and developed kidney damage. With a viable mouse model of XNDI that has all the key symptoms of the human disease, the scientists next turned to evaluating possible treatments. The V2R protein transmits signals within the cell through mediators known as G proteins, which act to increase intracellular levels of a molecule called cyclic AMP. Administration of a drug that activates the prostaglandin E receptor 4 (EP4), which also acts through G proteins and cyclic AMP, compensated significantly for the absence of V2R signaling. Mice that received a single injection of the drug showed an almost immediate decreased output of urine that was more concentrated, indicating that the drug was highly effective and quick-acting. Prolonged drug treatment was not associated with any obvious side effects. Moreover, kidney damage was halted in mice during extended drug treatment, while it worsened over the same period in animals that did not receive the drug. Together, these observations demonstrate that signaling through the EP4 receptors may represent a valid approach to ameliorate the symptoms of XNDI. These findings should stimulate the development of a new generation of drugs to treat this form of human nephrogenic diabetes insipidus.

Li JH, Chou CL, Li B, Gavrilova O, Eisner C, Schnermann J, Anderson SA, Deng CX, Knepper MA, Wess J: A selective EP4 PGE2 receptor agonist alleviates disease in a new mouse model of X-linked nephrogenic diabetes insipidus. J Clin Invest 119: 3115-3126, 2009.

KIDNEY TRANSPLANT RESEARCH

Living Kidney Donors Have Similar Long-Term Survival and Quality of Life as the General Population: Most kidney transplants involve organs from cadaveric donors; however, the demand for these organs far exceeds their supply. Therefore, some people opt to donate one of their two healthy kidneys, often to a sibling or other relative whose kidneys have failed. A recent study of nearly 3,700 people who donated kidneys between 1963 and 2007 found that people who choose to donate a kidney appear to have a normal life span, and that their risk of developing kidney failure is similar to that of the general population. Within a subset of donors who were studied more intensively, kidney function and high blood pressure were similar to the general population, and their quality of life was found to be excellent. Importantly, the researchers found no evidence of excess loss of kidney function over time in donors, some of whom donated kidneys 20 or more years ago.

Although the results of this study are good news for the participants, there are some important caveats. Potential kidney donors must meet strict selection criteria before they are allowed to donate. The relatively good health of the donors may explain at least part of the reason why their health and quality of life was found to be at least equal to, or better than, that of the general population. Additionally, in the past many volunteers donated a kidney at a relatively young age. In more recent times, however, the average age of donors has risen, and researchers and physicians will need to carefully monitor the health of these older volunteers. Additionally, participants in the current study were overwhelmingly Caucasian, and researchers do not know to what extent these findings can be extrapolated to kidney donors of other races and ethnicities. For example, African Americans have a much higher rate of diabetes, high blood pressure, and kidney failure than Caucasians, and it is possible they might be more likely to develop those conditions after donating a kidney. Further research will be necessary to better understand the long-term implications of kidney donation across a broad spectrum of the American population.

Nevertheless, the results of the current study indicate that there are few or no long-term detrimental health consequences for individuals who choose to donate a kidney. This finding may make potential donors more likely to donate a kidney, and have the consequence of increasing the supply of organs available for transplant.

Ibrahim HN, Foley R, Tan L, Rogers T, Bailey RF, Guo H, Gross CR, and Matas AJ: Long-term consequences of kidney donation. N Engl J Med 360: 459-469, 2009.

UROLOGY RESEARCH

Weight Loss in Overweight and Obese Women Reduces Urinary Incontinence: Researchers have recently reported that weight loss reduces urinary incontinence in overweight and obese women. An estimated 13 million Americans, most of them women, suffer from urinary incontinence. Women usually experience either "stress" and/or "urge" urinary incontinence. Stress urinary incontinence is the leakage of small amounts of urine during physical activity, such as coughing, sneezing, and exercising. Urge urinary incontinence is the leakage of large amounts of urine at unexpected times, including during sleep. Many women who have the disorder suffer in silence due to embarrassment. Obesity is an established and modifiable risk factor for urinary incontinence, but conclusive evidence for a beneficial effect of weight loss on urinary incontinence has been lacking.

The NIDDK's Program to Reduce Incontinence by Diet and Exercise (PRIDE) study recruited 338 obese and overweight women, who leaked urine at least 10 times per week, to determine whether a weight loss program could significantly reduce the frequency of urinary incontinence. The women were randomly assigned to one of two groups—one that participated in an intensive 6-month weight loss program of diet, exercise, and behavioral modification; or another that received information about diet and exercise, but no training to help them change their lifestyle. After 6 months, the investigators reported that women in the intensive group lost an average of 8 percent of their body weight (about 17 pounds) and reduced weekly urinary incontinence episodes by nearly one-half (47 percent). In contrast, women in the information-only group lost an average of 1.6 percent of body weight (about 3 pounds) and had 28 percent fewer episodes. Among women in the intensive treatment group, 41 percent achieved a clinically important reduction of at least 70 percent of weekly total incontinence episodes, whereas 22 percent of women in the information-only group achieved the same level of reduction. PRIDE provides high-level evidence that a behavioral intervention reduces urinary incontinence in overweight and obese women thereby permitting patients and their health care providers to make better informed and personalized treatment decisions to improve this disorder.

Subak LL, Wing R, West DS, Franklin F, Vittinghoff E, Creasman JM, Richter HE, Myers D, Burgio KL, Gorin AA, Macer J, Kusek JW, and Grady D for the PRIDE Investigators: Weight loss to treat urinary incontinence in overweight and obese women. N Engl J Med 360: 481-490, 2009.

A Potential Vaccine Approach for the Treatment of Urinary Tract Infection: Scientists have recently reported the successful use of a live, attenuated vaccine in mice to prevent bacterial infection in the bladder. Bladder and urinary tract infections (UTIs) are common, especially in women. About one-third of all women in the U.S. are diagnosed with a UTI by the time they reach 24 years of age—and many women suffer repeated UTIs. Most UTIs are caused by a common type of *Escherichia coli* (*E. coli*) bacterium. While antibiotic treatments are available, the rise of antimicrobial resistance among urinary tract invaders warrants improved prevention and treatment strategies.

The researchers hypothesized that use of attenuated (live but weakened) bacteria that stimulate the host immune response without causing disease may be beneficial in preventing or ridding the body of UTIs. A mutant strain of UTI-causing *E. coli* engineered to lack a gene important to the bacteria's virulence (*waaL*) appeared to be a strong candidate. To begin to assess whether the mutant *E. coli* may be clinically beneficial in protecting a host from UTI, the researchers performed a series of experiments testing its efficacy in rodent models. In these experiments, mutant *E. coli* were delivered into the bladders of uninfected mice as a potential vaccine; these mice, as well as mock-treated mice, were then challenged 14 days later with the normal, UTI-causing *E. coli*. The scientists found that mice inoculated with the mutant bacteria vaccine were protected from subsequent bladder infection. Importantly, the mutant bacteria were not themselves able to effectively infect the mouse bladders. Vaccination with the mutant *E. coli* strain also not only protected against infection by the original bacterial strain from which it was derived, but also protected against challenge from other, different strains of UTI-causing *E. coli*—indicating that this type of vaccine approach may provide broad clinical protection. While the exact mechanism for this protection is still under investigation, and additional studies will need to be performed to determine whether this live-attenuated vaccine may also hold promise for resolving recurrent UTIs in people, these findings are encouraging in the quest to find effective new UTI therapies.

Billips BK, Yaggie RE, Cashy JP, Schaeffer AJ, and Klumpp DJ: A live-attenuated vaccine for the treatment of urinary tract infection by uropathogenic Escherichia coli. *J Infect Dis 200: 263-272, 2009.*

Common Treatment for Chronic Prostatitis-Chronic Pelvic Pain Syndrome Fails To Reduce Symptoms: Scientists recently reported that a drug commonly prescribed for men with chronic prostatitis-chronic pelvic pain syndrome did not significantly reduce symptoms compared to a placebo control. Chronic prostatitis-chronic pelvic pain syndrome has no known cause and no uniformly effective therapy, and is the most common subtype of prostatitis seen by physicians. Men with this condition experience pain in the genital and urinary tract areas, lower urinary tract symptoms such as pain in the bladder area and during urination, and sexual problems that can severely affect their quality of life. More than one-half of primary care physicians prescribe alpha blockers, a class of drugs that relax the smooth muscle of the bladder and prostate, to treat the symptoms of chronic prostatitis-chronic pelvic pain syndrome. Some small randomized clinical trials have suggested that alpha blockers are effective in treating the symptoms of this condition. Because of the widespread use of these drugs, researchers recently examined the effectiveness of an alpha blocker in a larger randomized, placebo-controlled clinical trial in men with chronic prostatitis-chronic pelvic pain syndrome who had not previously been treated with this class of drugs.

A total of 272 men recently diagnosed with chronic prostatitis-chronic pelvic pain syndrome were randomly assigned to treatment for 12 weeks with the alpha blocker, alfuzosin, or a placebo. The NIH Chronic prostatitis-chronic pelvic pain syndrome Symptom Index score—a 0 to 43-point scale that measures severity of symptoms—was used to assess the effectiveness of alfuzosin versus placebo. The Index measures aspects of three important symptom areas associated with chronic prostatitis–chronic pelvic pain syndrome: pain, voiding problems, and negative effects on quality of life. After 12 weeks, no significant Index differences were observed between the two groups. Thus, the study showed that a drug commonly prescribed for men with chronic prostatitis–chronic pelvic pain syndrome was not effective in improving symptoms compared to placebo. The results of this study will inform future clinical trials. They may also impact patient care, sparing patients from an ineffective treatment for their condition.

Nickel JC, Krieger JN, McNaughton-Collins M, Anderson RU, Pontari M, Shoskes DA, Litwin MS, Alexander RB, White PC, Berger R, Nadler R, O'Leary M, Liong ML, Zeitlin S, Chuai S, Landis JR, Kusek JW, Nyberg LM, and Schaeffer AJ for the Chronic Prostatitis Collaborative Research Network: Alfuzosin and symptoms of chronic prostatitis-chronic pelvic pain syndrome. N Engl J Med 359: 2663-2673, 2008.

Improving Blood Stem Cell Transplantation Outcomes for Patients with Severe Sickle Cell Disease: Scientists have recently reported success with a modified bone marrow transplantation procedure to treat adult patients with sickle cell disease. Sickle cell disease is caused by a mutation in the beta globin chain of hemoglobin A, resulting in a chronic, often fatal, anemia. Under conditions of low oxygen, red blood cells become rigid and sickle-shaped, blocking blood vessels and causing severe pain. In the U.S., this genetic disease occurs predominantly in people of African descent, and is accompanied by episodic severe pain in the joints, leg ulcers, jaundice, organ damage, and other serious health conditions including, in rare cases, multi-organ failure. Mature red blood cells arise from stem cells. Transplantation of blood stem cells, obtained from bone marrow or another source such as umbilical cord blood, has been used to cure children with severe congenital anemias, such as sickle cell anemia. However, the medical procedures used for preparing patients for transplantation have thus far been too toxic to be used in adults.

A team of researchers developed a different regimen in preparation for blood stem cell transplantation, with the goal of making this treatment approach safer for use in adults. Bone marrow or other blood stem cell transplantation is typically preceded by destruction of the patient's own blood cells, to prevent immune reactions against the transplanted healthy donor cells and eliminate the disease-carrying blood cells. In this new regimen, instead of using chemotherapy to destroy the patient's bone marrow before infusing donor stem cells—as in the standard, prohibitively toxic procedure—the researchers used a low dose of radiation combined with two immunosuppressive drugs. This type of procedure is referred to as "non-myeloablative," meaning that it does not destroy the patient's own bone marrow. Rather, it is thought to create "space" for the donor stem cells to successfully engraft. After undergoing the non-myeloablative procedure, the patients, who all had severe sickle cell disease, were infused with peripheral blood stem cells from healthy sibling donors.

At a median follow-up of 2.5 years post-transplantation, the researchers reported that all 10 adult participants who had sickle cell disease were alive, and that 9 of them showed several reversed sickle cell diagnostic markers—including the return of hemoglobin levels to within normal range and the reduction of total amount of HbS protein to that seen in the donors. The results of this modified transplantation protocol strongly suggest that adult patients with sickle cell disease now have an additional option when considering how best to treat their condition.

Hsieh MM, Kang EM, Fitzhugh CD, Link MB, Bolan CD, Kurlander R, Childs RW, Rodgers GP, Powell JD, and Tisdale JF: Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med 361: 2309-2317, 2009.

BCL11A Regulates Production of Fetal Hemoglobin: Two research groups recently reported that the protein BCL11A acts to repress a gene encoding one of the two forms of globin in human fetal hemoglobin (also called "HbF"). HbF consists of four globin subunits: two "alpha globin" subunits and two "gamma globin" subunits. HbF is present during human fetal development but, owing to the shut-down of the genes encoding the gamma subunits, levels of HbF begin to decline after birth. The form of hemoglobin in children and adults contains a different type of subunit, called "beta globin," instead of the gamma subunits of HbF. Although HbF is virtually undetectable in most adults, people who have hemoglobin disorders such as sickle cell disease, which causes blockage of blood vessels by red blood cells that have formed a crescent-like shape, or beta-thalassemia, which results in reduced number and viability of red blood cells, sometimes retain varying levels of HbF after birth. This HbF can partially compensate for the defective or impaired function of adult hemoglobin in sickle cell disease or thalassemia, thereby ameliorating the clinical condition. Thus, prevention or reversal of the natural HbF decline in children and adults represents a potential strategy for the treatment of these disorders.

Previous studies had identified the protein BCL11A as a possible regulator of one of the globin genes, *HBG2*, which encodes the gamma subunit of HbF. Recently two independent research groups sought to confirm BCL11A's involvement in HbF regulation and determine its mode of action. Using cell culture systems, the scientists artificially increased or decreased the level of BCL11A protein and subsequently monitored the level of HbF or *HBG2* gene activity. High levels of BCL11A were found to decrease HbF levels. Under conditions of low to no BCL11A, HbF levels increased. Additional studies indicated that BCL11A directly acts to block HbF production by binding to specific sites near the *HBG2* gene, thereby preventing it from being turned on.

One of these research teams sought to determine the reason why humans and mice contain different globin subunits during early development. Whereas fetal human liver contains gamma globin subunits, fetal mouse liver contains beta globin. To investigate this difference, researchers sought to determine whether expression levels of BCL11A may be responsible for the different types of globin subunits between these two species. The results clearly show that BCL11A is present in much higher levels in fetal mouse liver than fetal human liver, accounting for the corresponding lack of gamma globin in the fetal mouse tissue.

These results confirm and expand scientists' understanding of the role of BCL11A in the regulation of HbF. Furthermore, this research also provides a potential therapeutic approach for HbF reactivation in patients with hemoglobin disorders.

Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, Mikkola HKA, Hirschhorn JN, Cantor AB, and Orkin SH: Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science *322: 1839-1842, 2008.*

Chen Z, Luo HY, Steinberg MH, and Chui DH: BCL11A represses HBG transcription in K562 cells. Blood Cells Mol Dis *42: 144-149, 2009.*

Sankaran VG, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, Fujiwara Y, Ito M, Groudine M, Bender MA, Tucker PW, and Orkin SH: Developmental and species-divergent globin switching are driven by BCL11A. Nature *460: 1093-1097, 2009.*

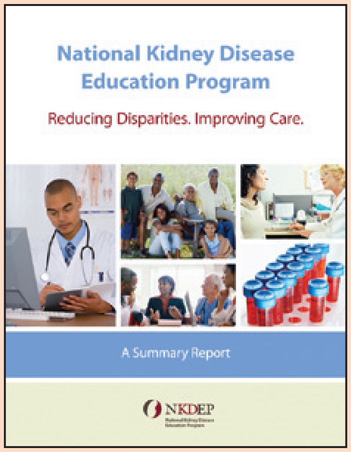
Blood Stem Cell Development with Implications for Tissue Regeneration: Scientists have recently identified key factors that interact to regulate stem cell development and tissue regeneration. Therapies to regenerate diseased or injured organs will benefit from an in-depth knowledge of how stem cells and progenitor cells develop into complex tissues and organs. Previous research has implicated several factors in the regulation of blood (hematopoietic) stem cell formation and self-renewal. These include prostaglandin E2 (PGE2) and a series of factors known as the WNT signaling pathway. However, the exact mechanism by which these factors exert their effects on hematopoietic stem cells remained unknown.

Beginning with the zebrafish model system, investigators showed that PGE2 directly increased WNT pathway activity within embryonic blood stem cells, leading to the formation of greater numbers of these stem cells. Exploring the underlying mechanisms, the researchers found that PGE2 helps stabilize an important component of the WNT pathway. In addition to demonstrating the importance of these factors to embryonic blood stem cell development, the scientists investigated a potential role for PEG2 and the WNT pathway in adult animals. By studying zebrafish whose blood stem cells had been destroyed by irradiation, the researchers discovered that these factors also work together to promote regeneration of new blood stem cells in adult fish. Further studies revealed that PGE2 and WNT work together to promote formation of embryonic and adult blood stem cells in mice—evidence that these pathways also interact in mammals. Beyond blood cells, the PGE2/WNT interaction was also shown to be required for liver regeneration in both fish and mice. These results illuminate PGE2 and the WNT pathway as powerful regulators of cellular regeneration in the body. Future research efforts will delineate the feasibility of delivering appropriate levels of PGE2 to damaged tissues to promote WNT-dependent cellular regeneration in a precise and controlled manner to preempt further injury and restore health.

Goessling W, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, Weidinger G, Puder M, Daley GQ, Moon RT, and Zon LI: Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell *136: 1136-1147, 2009.*

National Kidney Disease Education Program's Efforts To Improve Treatment Practices

An estimated 23 million Americans may have chronic kidney disease (CKD)[1] and, according to the NIDDK-supported U.S. Renal Data System, more than 500,000 patients are either on kidney dialysis or living with a kidney transplant.[2] Patients with CKD are at increased risk for kidney failure. It is estimated that treating the number of people with kidney failure, also called end-stage renal disease (ESRD), through dialysis or kidney transplantation costs U.S. taxpayers approximately $24 billion each year. ESRD is an enormous public health problem that disproportionately affects minority populations.



The NIDDK's National Kidney Disease Education Program (NKDEP) is helping to address these issues. This educational program seeks to raise awareness of the seriousness of kidney disease, the importance of testing people at high risk—those with diabetes, high blood pressure, cardiovascular disease, or a family history of kidney disease—and the availability of treatment to prevent or slow kidney failure. The progression from CKD to kidney failure can be prevented or delayed if it is detected and treated early enough. The NKDEP underscores that effective treatments and management strategies for kidney disease exist, yet are being underutilized.

Kidney disease is identified based on decreased kidney function or evidence of kidney damage, usually proteinuria. Kidney function is assessed through the estimated glomerular filtration rate, or eGFR. This value is derived from a mathematical formula that takes into account several factors that impact creatinine production, including age, gender, and race. Creatinine is a waste product in the blood created by the normal breakdown of muscle cells during activity. When kidneys are not working well, creatinine levels build up in the blood. Protein is the most common sign of kidney damage. Protein does not normally pass through the kidney filter into the urine. Along with eGFR, providers can measure the amount of the protein albumin in the urine (UA) to detect and plan treatment for chronic kidney disease. However, there are several issues related to standards for the measurement and reporting of UA that have made it difficult for health care providers to use test results effectively to inform treatment decisions and monitor patients' kidney health. As a result of a March 2007 meeting bringing together members from NKDEP and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), several working groups have been formed to study the biological variability of UA and develop appropriate measurement procedures.

The NKDEP has recently released a summary report that highlights major efforts to address chronic kidney disease disparities and improve care. The report can be found at: *http://nkdep.nih.gov/resources/NKDEP_Summary_Report_508.pdf*

For NKDEP educational materials for patients, health care providers, and laboratory professionals, please visit *www.nkdep.nih.gov* or call 1-866-4 KIDNEY (1-866-454-3639).

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[1] *Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, and Coresh J, for the Chronic Kidney Disease Epidemiology Collaboration: A new equation to estimate glomerular filtration rate. Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2009 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the U.S., National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2009.*

STORY OF DISCOVERY

The Promise of Induced Pluripotent Stem Cells

The NIDDK conducts and supports basic and clinical research on many of the most serious diseases affecting public health. One promising approach to prevent or treat a variety of diseases is in the recent research development of induced pluripotent stem (iPS) cells. Pluripotency is defined as the ability of stem cells to give rise to most of the various cell types in the body. In promising research, scientists are investigating the robustness of these cells as research tools, to advance understanding of disease development, and as potential sources of cells for regenerative therapies for patients.

Setting the Stage

The study of embryonic stem cells established a foundation on which to understand the properties of self-renewal and pluripotency. In 1981, the first mouse embryonic stem (ES) cell lines were isolated from embryos and grown in culture. These cells were among the first isolated cells to be shown to have the two key characteristics of embryonic stem cells: (1) They could grow and divide for long periods of time in an undifferentiated state (self-renewal)—that is, generating new stem cells, rather than specific cell types; and (2) they could also differentiate, or mature, into the many tissue- or organ-specific cell types. Human ES cell lines were established 17 years later and displayed similar properties to mouse ES cells with respect to their ability to both continually divide to form new daughter stem cells, and to develop into many different cell types.

While characterizing the transition from human ES cells into more mature cell types, NIDDK-supported researchers discovered that some genes, including *Oct4*, were turned off—suggestive of a collection of genes required for the indefinite renewal of ES cells, but no longer needed when the cell differentiates. This finding provided insight into a subset of genes that may have relevance to the renewal capability of other cell types.

Human iPS Cells—A Beginning

Building on a landmark study in mouse cells by researchers in Japan, NIDDK-supported scientists showed that differentiated skin cells derived from a human ES cell line could also be genetically "reprogrammed" to revert back to an ES cell-like state if new copies of four specific genes were introduced into the cells. The cell's own native copies of these four genes had been shut down when the cells differentiated. The scientists had engineered the new gene copies to be active and lead to production of the proteins they encoded in the differentiated cells. Once these proteins were produced in the cells, they caused the cells to be reprogrammed and to return to a stem cell-like state. Of the four proteins, one was Oct4 (encoded by the *Oct4* gene), which was known from previous research to be present in self-renewing ES cells and not in differentiating cells, as described above. The others were genes called *Klf4*, *Sox2*, and *Myc*. The reprogrammed human skin cells, or iPS cells, regained characteristics very closely resembling those of ES cells—that is, the four genes induced pluripotency. The ES-like characteristics included cell expansion essentially without limit; cell morphology (appearance) similar to ES cells; certain types of DNA modifications (called methylation) in ES cell-like patterns; and formation, in immuno-compromised mice, of a particular type of tumor observed in ES cell experiments. Remarkably, the researchers were also able to generate iPS cells from more developmentally advanced cells—including adult skin cells—by introducing two additional genes. This was quite an achievement as there had been a general belief that mature cells, such as adult skin cells, were incapable of reverting back to a less differentiated state. These findings also suggested that there may be a hierarchy of cells within tissues—some of which can be more easily reprogrammed than others. NIDDK-supported scientists have recently reprogrammed human blood cells using the same original cocktail of *Oct4*, *Sox2*, *Klf4*, and *Myc*. This finding represents an important advance as an initial strategy to generate iPS cells to correct for diseases specific to the blood system. Importantly, a number of laboratories have been able to successfully produce iPS cells by the method of introducing a defined set of genes.

Interestingly, a high level of the reprogramming proteins need to be present for only a short period of time to initiate the genetic program of converting the mature cell to an iPS cell. During this transition, the cell begins to increase the level of additional proteins while at the same time decreasing the level of the reprogramming proteins.

Reprogramming adult human cells would not have been possible without years of prior research studying the properties of human ES cells. Two fundamental factors critical to the development of human iPS cells are based on the knowledge gained from studying human ES cells: (1) knowledge of "stemness" genes (such as *Oct4*) whose expression or repression is essential to maintain pluripotency; and (2) the delineation of conditions necessary to propagate the cells in laboratory dishes.

Potential Uses of Human iPS Cells

NIDDK-supported scientists subsequently generated iPS cell lines from patients with several different genetic diseases and disorders, providing a valuable resource for the scientific research community. These iPS cell lines were derived from cells from patients with Parkinson's disease, type 1 diabetes, Huntington's disease, Down syndrome, severe combined immunodeficiency, Gaucher's disease, forms of muscular dystrophy, and other diseases. Comparing cells differentiated from these disease-specific iPS cell lines to cells from healthy individuals may provide insights into the development and progression of these diseases. The iPS cell lines may also be useful in the laboratory to screen new candidate therapeutic agents. Additionally, with further research progress, human iPS cells might be used one day in transplantation medicine. For example, mature cells taken from a patient could potentially be reprogrammed into iPS cells and, if necessary, a defective gene corrected. Transplanting these cells back into the patient would avoid the rejection by the immune system that occurs when tissue or organs from a different person are used for transplantation. Lack of immune rejection would also negate the need for immuno-suppressive drug therapy, which carries adverse side effects. It is hoped that transplanted iPS cells might in the future be used to treat, and perhaps cure, several diseases, such as sickle cell anemia, type 1 diabetes and its complications, and liver disease.

Challenges

Modified viruses are currently used to introduce the reprogramming factors into adult cells. This process would need to be replaced with safer methods before iPS cells could be used for treatments for humans. In animal studies, the viral "vectors" are known to randomly incorporate into the genome and sometimes cause cancer. Recent NIH-supported studies have reported using alternative methods to obtain iPS cells. One such approach used a modified viral vector which can be turned on for a specific period of time to produce the necessary proteins to initiate the reprogramming and then subsequently removed almost completely from the genome. A second approach utilized a protein expression system that does not integrate into the genome and, hence, essentially eliminates the possibility of causing a genetic mutation.

The process of making iPS cells is slow and inefficient. Evidence accumulated to date suggests that the reprogramming process is influenced by the differentiation state of the target cell. During normal differentiation, as the cell matures from an early to late stage, the genome acquires modifications in the form of DNA methylation as well as modifications of proteins called histones, which associate with and package DNA. For example, several regions of DNA that help regulate gene activity are unmethylated in early-stage cells but densely methylated in later-stage cells. Ongoing studies are investigating the use of chemicals to inhibit the methylation and histone modifications found in mature cells.

Ongoing studies also are examining the possibility of using a chemical-only approach to reprogram mature cells to iPS cells. Potentially, this means that chemicals would directly increase the levels of reprogramming proteins—thus avoiding the use of viral vectors.

Looking to the Future

Cautious optimism describes the eventual use of iPS cells for experimental models of disease, as targets in drug screening studies, and as sources for regenerating tissue. As described here, knowledge gained from studying embryonic stem cells sets the stage for the development of iPS cells. The derivation of iPS cells has led—and continues to lead—to a cascade of exciting and unexpected findings with broad implications for improving health.

Muscle Wasting in Kidney Disease and Other Conditions

Dr. William Mitch

Dr. William Mitch is the Gordon A. Cain Professor of Medicine and Director, Division of Nephrology, at Baylor College of Medicine in Houston, Texas. He received his M.D. from Harvard Medical School. Dr. Mitch is currently an NIDDK Advisory Council member and has received a MERIT award from the Institute. He is recognized as one of the world's experts on the care of patients with hypertension and chronic kidney disease, using dietary methods to protect the kidneys. In recognition of his substantial research contributions in nephrology (kidney disease) and sustained achievements in academic medicine, Dr. Mitch was awarded the prestigious John P. Peters Award in 2009 by the American Society of Nephrology. Dr. Mitch's distinguished career has largely focused on understanding the mechanisms that regulate protein metabolism, placing emphasis on how kidney disease changes protein metabolism. He has published over 250 papers. The following are highlights from the scientific presentation that Dr. Mitch gave to NIDDK's Advisory Council in February 2009.

Understanding the processes that cause the loss of muscle mass may lead to novel therapies for kidney failure, diabetes, sepsis, certain kinds of cancers, and other conditions in which muscle wasting occurs. Such knowledge also could help clinicians identify patients at greatest risk for loss of muscle mass.

The body's ability to maintain skeletal muscle tissue levels can go awry under conditions of extended bed rest, fasting, space flight, critical illness, or severe injury. Beginning with an example of the effects of bed rest, Dr. Mitch described a recent study that assessed the impact of extended bed rest on lean body mass (skeletal muscle) in healthy young and older adults. He observed that older participants lost more skeletal muscle during 10 days of bed rest than did younger participants after 28 days, suggesting that older adults requiring hospitalization for health related issues are at increased risk for significant loss of skeletal muscle.

End-Stage Kidney Disease (Kidney Failure) and Skeletal Muscle Loss

Dr. Mitch next recounted an earlier clinical finding on kidney disease that had sparked his own research interest. The 1980 study assessed the nutritional status of kidney failure patients who were undergoing dialysis, as compared to individuals with normal kidney function. When the kidneys are not functioning effectively, urea accumulates in the blood, a condition known as uremia. In some patients, this condition may progress to irreversible kidney failure, requiring dialysis—or a kidney transplant—to cleanse the body of urea and other waste products normally excreted in the urine by healthy kidneys. The results of the study were striking, showing that patients with uremia were more likely to have reduced body weight, body fat, and skeletal muscle mass in the upper, non-dominant arm. These findings confirmed what had been observed frequently for this population of patients—that wasting is common.

Dr. Mitch and his team then studied an animal model of kidney failure to investigate potential causes of muscle wasting. Because kidney failure had previously been associated with acidosis (high levels of acid in the blood and tissues), the scientists sought to determine whether acidosis promotes loss of muscle protein. When they evaluated the animals, they found that those with decreased kidney function lost muscle mass (protein) at a much greater rate than control animals. However, when fed bicarbonate, which can help maintain proper acid-base balance in the body, the animals with impaired kidney function no longer lost muscle protein; indeed their net muscle protein loss resembled that seen in the control animals. Thus, these experiments showed that muscle protein loss in an animal model of kidney failure is influenced by acidosis that in turn can be corrected by administering bicarbonate.

What Drives the Loss of Lean Body Mass?
In further describing the link between acidosis and muscle mass, Dr. Mitch noted several studies by others. For example, when children with inherited conditions such as renal (kidney) tubular acidosis are fed bicarbonate, they grow more normally. In addition, people with kidney disease given bicarbonate do not break down more protein than usual, thereby maintaining lean body mass at normal levels. Conversely, when an acidic solution is delivered intravenously to healthy adults, they begin to break down more protein than usual.

What is the signal or trigger that causes muscle protein loss? In further studies of metabolic acidosis in uremic animals, Dr. Mitch and his team found that the pH, a measure of acid-base level, within cells was normal, not acidic. They then explored another potential trigger. Conditions such as sepsis, trauma, and inflammation, which stimulate muscle breakdown, share a common feature—insulin resistance, a state in which the body produces the hormone insulin but is unable to use it properly. Dr. Mitch and his colleagues hypothesized that defects in signaling by insulin and insulin growth factor-1 (another hormone) may cause loss of muscle protein.

Type 2 diabetes is characterized by insulin resistance. Experiments were designed to determine whether insulin resistance causes muscle breakdown in type 2 diabetes using the *db/db* mouse model. Three different types of muscle (soleus, extensor digitorum longus, and plantaris) from the mice with diabetes were all shown to have higher rates of protein degradation compared to their respective muscle type in normal mice, demonstrating an association between insulin resistance and increased muscle protein loss.

How Does Impaired Insulin Signaling Stimulate Protein Breakdown?
When cells break down proteins, the process most often used is called the ubiquitin-proteasome pathway. Dr. Mitch and his team speculated that this pathway is also involved in protein degradation stimulated by impaired insulin signaling. The pathway begins with the addition of several ubiquitin molecules to proteins to tag them for degradation. Tagged proteins are then recognized by a large structure, the proteasome, which chops up the proteins and releases the ubiquitin molecules to be recycled. Dr. Mitch provided evidence that the levels of ubiquitin and atrogin-1—the factor that joins ubiquitin to proteins destined for degradation—may be elevated in an experimental model of diabetes compared to control animals. Additional experiments showed that an inhibitor of proteasome function can block the increase in protein degradation caused by insulin resistance. Therefore, Dr. Mitch and his colleagues predicted that in chronic kidney disease and other diseases associated with muscle wasting, the ubiquitin-proteasome pathway is in an activated state, which contributes to increased muscle protein breakdown. He also outlined a cascade of molecular events through which impaired insulin signaling ultimately drives another cellular factor to activate the gene for atrogin-1, which in turn helps accelerate ubiquitin-proteasome activity and, hence, muscle protein degradation.

How Are Larger Muscle Protein Structures Degraded?
The myofibril is the contractile structure in skeletal muscle, and is composed mainly of the proteins actin and myosin. Reconstituted in a test tube, the

ubiquitin-proteasome system quickly degrades actin and myosin, but does not break down larger complexes of these two proteins—actomyosin or myofibrils. Metabolic acidosis places stress on cells. Dr. Mitch and his team were aware that other forms of cellular stress, for example inflammation, lead to activation of caspase-3, a type of protein that can cleave other proteins. They thus hypothesized that perhaps caspase-3 plays a role in the initial steps of muscle breakdown.

To determine whether caspase-3 is capable of the initial cleavage of muscle protein, Dr. Mitch and his colleagues added it to the actin-myosin complex (actomyosin) in a test tube. They found that caspase-3 cleaved actomyosin to generate native actin, as well as a smaller fragment of actin, which they referred to as a "14 kD actin fragment." Additional investigation revealed that the 14 kD fragment could be degraded further by the ubiquitin-proteasome system.

The 14 kD Actin Fragment as a Marker in Experimental Models and Human Disease

Dr. Mitch and his team next examined caspase-3's possible role in the initial step of increased muscle protein loss under disease conditions. Muscle tissue from animal models of acute diabetes or chronic uremia was compared with muscle from normal animals, and was found to have higher levels of 14 kD actin fragment. Additionally, more 14 kD actin fragment was detected in muscle from patients with degenerative hip disease—who are generally less mobile—and the amount of the 14 kD fragment in the muscle correlated with the measured rate of protein degradation. Thus, caspase-3 generates a characteristic 14 kD actin fragment during conditions of muscle wasting, and this fragment could be used as a biomarker of muscle protein loss.

Dr. Mitch's team then investigated whether this actin fragment reflects muscle wasting in another disease, and whether it may help in evaluating potential therapies. The impact of two types of exercise on the amount of 14 kD actin fragment in thigh muscle was evaluated in patients undergoing hemodialysis. After 18 weeks of endurance exercise, there was a decrease in the amount of actin fragment when compared to the pre-exercise period. Strength training, however, did not reduce the levels of the actin fragment after 18 weeks. These findings suggest that endurance training decreased muscle protein breakdown in patients undergoing hemodialysis, and that analysis of the actin fragment may be a useful way to assess protein degradation.

Caspase-3 Stimulates Proteasome Activity

The proteasome complex is responsible for most of the protein degradation that occurs within all cells. It includes a central core, which degrades proteins, surrounded by numerous other subunits. Dr. Mitch and colleagues suspected that these other subunits might regulate the overall activity of the proteasome.

In a series of experiments with different muscle cells, Dr. Mitch and his team found that several of the proteasome's subunits can be cleaved within the cell to increase the activity of the proteasome to degrade muscle proteins. Interestingly, these proteasome subunits are cleaved by caspase-3, the same factor that also cuts muscle proteins to prepare them for further degradation by the proteasome. To identify which subunits of the proteasome might be susceptible to cleavage by caspase-3, Dr. Mitch and his colleagues turned to a cell culture system containing either fully formed, mature muscle cells (myotubes), or immature muscle cells (myoblasts). Through experimental modulation of caspase-3 activity and analysis of the effects on various proteasome subunits, they discovered that caspase-3 cleaves different subunits in different types of muscle cells. In the immature cells, caspase-3 cuts one of the subunits and reduces proteasome activity. However, in mature muscle cells, caspase-3 cleaves different subunits, leading to an <u>increase</u> in proteasome activity, and thus to more muscle protein degradation.

Conclusions

Dr. Mitch's experiments have elucidated mechanisms underlying muscle wasting in chronic kidney disease and other conditions. Defective signaling by the hormones insulin and insulin growth factor-1 leads ultimately to increased activity of cellular components that degrade muscle proteins: caspase-3, the proteasome, and another factor that tags proteins for degradation. Caspase-3 both initiates muscle protein breakdown and activates proteasomes. Interventions designed to restore insulin signaling may also slow the loss of muscle protein. This research also revealed the potential beneficial use of bicarbonate to slow the loss of muscle protein in people with kidney disease. For patients undergoing hemodialysis, endurance training was shown to decrease muscle protein breakdown and thereby improve the nutritional status of the patient. Furthermore, Dr. Mitch and his colleagues have identified a marker, the 14 kD actin fragment, that has the potential to aid in the diagnosis of muscle protein wasting as well as being an indicator to assess therapeutic interventions to reverse the condition.

Dr. Mitch acknowledged the contributions of scientists who worked with him on these studies: Drs. Jie Du, Liping Zhang, Daniel Hu, S. Woo Lee, In Hee Lee, and Vik Rajan from Baylor College of Medicine; Drs. S. Russ Price and Xiaonan Wang from Emory University; Dr. A. Ferrando from the University of Arkansas School of Medicine; and Drs. Fred Goldberg and Stewart Lecker from Harvard Medical School. Dr. Mitch also indicated his appreciation of NIDDK support over the years.

A Critical Developmental Switch Controls the Onset and Progression of Cyst Formation in Polycystic Kidney Disease

Dr. Gregory G. Germino

Dr. Gregory Germino was named the Deputy Director of NIDDK in June 2009. Prior to joining NIDDK, Dr. Germino, a highly regarded physician-scientist, was a Professor in the Department of Medicine and Department of Molecular Biology and Genetics at The Johns Hopkins University School of Medicine. He received his M.D. from the University of Chicago, completed his residency in internal medicine and nephrology at Yale University, and conducted a clinical fellowship at Oxford University. Dr. Germino is a world-renowned expert in inherited kidney disease and has made seminal contributions to understanding the genetic origins of polycystic kidney disease. His research accomplishments have been recognized by several honors, including an NIH Physician-Scientist Award (1988-1993) and an NIH MERIT award (2000). At the September 2009 meeting of the NIDDK Advisory Council, Dr. Germino presented recent advances on the molecular factors contributing to the onset and progression of polycystic kidney disease; the following are highlights from his presentation.

Kidney Structure and Function

The kidneys are a pair of vital organs that keep the blood clean and chemically balanced. Blood is processed in the kidneys by millions of functional units called nephrons, where each nephron contains a filtration unit—known as a glomerulus—that is connected to a tube (or tubule) for collecting the filtered waste products. As filtered waste is collected, the tubules measure out the amounts of different chemicals present to ensure that the body maintains the right balance that is necessary for life. This important function requires tubules to have distinct cell types properly positioned with the correct orientation. If the tubule structure is compromised in some way, kidney function could be seriously impaired.

Polycystic Kidney Disease

Polycystic kidney disease (PKD) is an inherited disorder in which abnormal tubule structure leads to the formation of numerous fluid-filled cysts in the kidney. These cysts can slowly increase the mass of the kidneys and impair kidney function, leading to kidney failure. About half of people with the most common form of PKD progress to irreversible kidney failure, ultimately requiring either a kidney transplant or dialysis to survive.

In the U.S., an estimated 1 in every 1,000 people have PKD, and it is the fourth-leading cause of kidney failure. Although people with this disease have a number of options for managing pain, preventing infection, and slowing the decline of kidney function, there are no effective therapies at present to target the underlying cause of PKD progression and reverse the loss of kidney function.

Genetic Cause of PKD

Because PKD is an inherited disorder, scientists and physicians have the unique opportunity to understand the disease by studying the genetic mutations that cause it. The most common form of PKD, known as autosomal dominant PKD (ADPKD), is associated with inherited mutations in one of two genes—*PKD1* or *PKD2*. Although mutations in *PKD1* account for 85 percent of ADPKD cases, the resulting disease is clinically indistinguishable from ADPKD caused by inherited mutations in *PKD2*.

Typically, if a disease is "autosomal dominant," only one abnormal copy of a gene needs to be inherited to cause the disease. That is, of the two copies of the gene that a person inherits—one from each parent—the abnormal one "dominates." In the case of autosomal dominant PKD, however, the genetics appear to be somewhat more complicated. Although an individual with ADPKD may inherit one mutated copy of *PKD1*, for example, along with one normal copy of the gene, it is not until this normal copy acquires a mutation that cyst formation is "triggered" and disease onset begins. These acquired mutations, which occur by chance in the genes of individual cells, are called somatic mutations, to distinguish them from mutations that are inherited. (The situation is similar for *PKD2* gene mutations.) They are also referred to as "second-hit" mutations, with the first mutation being the inherited one.

By analyzing the genetic composition of kidney cysts, researchers have found that these "second-hit" mutations occur rather frequently. In addition, experiments in genetically-engineered mice with PKD mutations have shown that the severity of cystic disease is primarily determined by how quickly the normal gene copy acquires new, non-inherited mutations. Because different types of mutations impair PKD gene function to different extents, researchers have put forth a model in which the combined effects of the inherited and non-inherited mutations have to reach a certain "threshold" to trigger cyst formation.

PKD1 and _PKD2_ Gene Products and Disease

What do the *PKD1* and *PKD2* genes do, and how do they contribute to PKD onset and progress? The *PKD1* and *PKD2* genes provide the genetic information that is used to make two cellular proteins, polycystin-1 (PC-1) and polycystin-2 (PC-2), respectively. The PC-1 and PC-2 proteins are known to be important in determining the types of cells that are formed in kidney tubules and are critical for tubule development and structure.

PC-1 and PC-2 are large proteins that sit on the surface of tubule cells and form a "receptor-channel complex." A large portion of PC-1 extends outward from the cell surface and acts as an antenna (or receptor) to sense (receive) chemical, cellular, or mechanical stimuli. Detection of these stimuli by PC-1 is then coupled—or transmitted—to PC-2, which acts as a channel to let calcium, an important cellular messenger, flow into the cell. It is believed that this sense-and-respond signaling is important in mediating interactions between cells, as well as interactions between a cell and the matrix of molecules lining the extracellular surface. In addition, the PC-1/PC-2 receptor-channel complex is also found on hair-like protrusions, known as cilia, which extend from the cell surface into the interior of tubules, where liquid flows. It is on the surface of cilia where PC-1/PC-2 sends signals back to the cell in response to either the mechanical forces it feels while fluid flows through the tubule or to some other undefined trigger. Disruption of normal cilia signaling through mutations in *PKD1* or *PKD2* that alter PC-1/PC-2 function may be an underlying contributor to PKD.

Life Stage Determines Response to Loss of _PKD1_

Although the PC-1/PC-2 complex is important for establishing tubule structure during kidney development, researchers are not certain what role PC-1/PC-2 plays in maintaining tubule structure in the adult (developed) kidney. As such, an important clinical question relates to when *PKD1* and *PKD2* are required for kidney function: Are mutations that occur in these genes during adulthood sufficient for triggering cyst formation, or do these inactivating mutations (and cyst formation) need to occur at an earlier life stage when the kidneys are still developing? To address this important question regarding the onset of PKD, Dr. Germino and his colleagues have generated genetically-engineered mouse models of PKD in which they can turn off the *PKD1* gene in a controlled manner at various time points. By looking at how "knocking out" *PKD1* during different stages of development affects cyst formation, they can evaluate the role of *PKD1* in the developing and adult kidney.

Initially, Dr. Germino's team looked at the effect of *PKD1* inactivation in newborn, adolescent, and adult mice. When the researchers inactivated *PKD1* in mice 2 days after they were born, the mice developed severely cystic kidneys quickly, within a couple of weeks. In contrast, if the researchers waited until the mice were adolescents or adults before inactivating *PKD1*, it took at least 5 months for cysts to develop. Since kidneys are fully developed in adolescents and adults and *PKD1* inactivation at that point still results in cyst formation, these results indicate that *PKD1* is important for maintaining normal tubule structure during adulthood. Interestingly, however, the progression of cyst formation was unexpectedly different following *PKD1* inactivation during different life stages.

To better define the developmental period that controls the differential response to loss of *PKD1* function, the researchers inactivated *PKD1* in mice at various intervals between 2 and 21 days after birth. If *PKD1* was inactivated when the mice were 12 days old or younger, their kidneys developed cysts very rapidly—within a couple of weeks, as was seen with the mice whose PKD genes were inactivated just 2 days after birth. Remarkably, when *PKD1* was inactivated 14 days or more after birth, the kidneys of the mice all appeared normal for 3 months but then developed late-onset cystic disease by 6 months. Examining the molecular and genetic differences that may contribute to this difference in timing of cyst formation, the researchers found that the rate of cellular growth and division in the kidneys drops abruptly at 16 days after birth. Paralleling this change in cellular growth, the cells underwent dramatic changes in genetic activity between 12 and 14 days after birth, with over 800 different genes becoming more or less active during this period. These results identify an important developmental switch that is being triggered during this critical time frame.

From these experiments in mice, Dr. Germino proposed that, in addition to the rate of acquiring mutations that inactivate the once-normal copies of the *PKD1* gene, the timing of *PKD1* inactivation is another critical factor for determining the rate of cyst growth and disease onset.

Future Directions and Clinical Implications

At this point, it is not exactly clear what accounts for the life-stage specific response to *PKD1* inactivation. Although the rate of cellular growth drops abruptly at day 16, the relationship between cyst formation and cell growth is rather complicated; the decline in cellular growth may reflect completion of the final stages of tubule maturation. In addition, the rate of disease progression does not appear to be due to disruption of PC-1/PC-2 as the cilia's mechanical sensor. Although flow sensing may be important during kidney development, the long interval between loss of PC-1 and cyst formation in adults suggests that a dynamic process like fluid flow would not be the cause of disease onset. Further experiments will be needed to fully understand the role of PC-1 at different life stages—such as its role in orienting cells after cell division—and how it contributes to the late-onset of cyst formation following inactivation in adults.

Understanding why *PKD1* inactivation in adult mice leads to late-onset cyst formation has important clinical implications for human disease. Dr. Germino's studies suggest that the underlying molecular causes may differ for early-onset and late-onset PKD, with the late-onset mouse model reflecting the gradual onset and slow progression of ADPKD in humans. To date, however, most testing of potential therapies has been done in mouse models with relatively rapid rates of disease onset and progression. Thus, it is not clear whether such studies would apply to human disease. As there are currently no therapeutic options available, the late-onset disease models developed by Dr. Germino may provide a better tool for testing new therapies in ADPKD.

PATIENT PROFILE

Janet Colardo

Benefits of Weight Loss in Reducing Urinary Incontinence in Overweight or Obese Women

Janet Colardo

An estimated 13 million Americans, most of them women, suffer from urinary incontinence (UI). Encouraging research sponsored by the NIDDK and the NIH Office of Research on Women's Health has recently shown, however, that women who are overweight or obese can significantly reduce their episodes of UI by losing weight.

Fifty-three-year-old Janet Colardo says she has always been overweight, and although she might not have thought of it as UI, she cannot remember a time when she didn't leak urine involuntarily, especially when someone or something made her laugh. "I've always been one of those pear-shaped people," says Janet. "In high school, when my classmates squeezed into designer jeans, I just couldn't do it." Janet also says that she was always asking her teachers for permission to go to the bathroom.

For decades Janet considered her urinary condition normal. "I figured it was just who I was." Then in 2006, she noticed an ad in her local newspaper for a study on weight loss and UI. The timing was perfect. "One of my sons was getting married, and I wanted to lose weight for the wedding," Janet says. "That was a big motivator for me. I figured if I could lose weight and learn how to control my bladder at the same time, all the better." Janet was interviewed and accepted into the study.

Reducing urinary incontinence can now be added to the extensive list of health benefits of weight loss, according to a clinical trial funded by the NIDDK and the NIH Office of Research on Women's Health.

PRIDE

The study Janet volunteered to participate in was called the Program to Reduce Incontinence by Diet and Exercise, or PRIDE.

Conducted in Birmingham, Alabama, and Providence, Rhode Island, PRIDE researchers recruited a total of 338 overweight and obese women who experienced UI episodes at least 10 times per week. The women were randomly assigned to either an intensive 6-month weight-loss program of diet, exercise and behavior modification or to a group that received information about diet and exercise, but no training to help them change habits.

PRIDE researchers reported that women in the intensive weight-loss group lost an average of 8 percent of their body weight (about 17 pounds) and reduced UI episodes by nearly one-half (47 percent). In contrast, women in the information-only group lost an average of 1.6 percent of body weight (about 3 pounds) and had 28 percent fewer episodes. Further analysis of the data showed that 41 percent of the women in the intensive weight-loss group achieved a clinically relevant reduction of at least 70 percent of total incontinence episodes per week, whereas only 22 percent of women in the information-only group achieved the same level of reduction.

PRIDE showed conclusively that weight loss has a significant, positive impact on reducing UI in women. This finding may help motivate weight loss, which has additional health benefits for those who are overweight or obese. One such benefit is prevention of type 2 diabetes. In fact, the intensive weight-loss intervention in PRIDE was modeled after the lifestyle interventions in the Diabetes Prevention Program (DPP) and the ongoing Look AHEAD (Action for Health in Diabetes), two NIDDK-sponsored clinical trials of people who are at risk for or who have type 2 diabetes, respectively.

Participating in the Study

Janet entered the program weighing 193 pounds and completed it 6 months later weighing in the mid 170s. "I went right down in my weight," says Janet, who is 5 feet, 7 inches tall. She also reports that her incidence of UI dropped significantly.

"The PRIDE staff was extremely supportive. They provided us with all the information and tools we needed, and it was up to us to put them to good use."

For example, each participant in Janet's group was informed about proper eating habits and was issued a journal to document daily caloric intake. They also were shown how to properly perform Kegel exercises to strengthen their pelvic floor muscles, which when weakened as a result of excessive weight or other conditions (*e.g.*, pregnancy, childbirth, and aging) can lead to UI.

"The PRIDE staff was extremely supportive. They provided us with all the information and tools we needed, and it was up to us to put them to good use."

Janet admits that she never really exercised before entering the study. However, she says she now has a greater respect for the many benefits of exercise and living a healthy lifestyle, which she says the PRIDE staff instilled in her. To encourage more daily activity, Janet and her fellow participants were given pedometers to measure the distance they walked each day.

Other NIDDK-sponsored research has shown the link between excessive body weight and diabetes. Since the program ended, Janet says she has been dealing with endocrine problems that are making it difficult for her to lose weight, and she has put on some of the weight she lost. Fortunately, however, she hasn't been diagnosed with diabetes.

"If you tend to be on the heavy set side, you're always struggling to lose weight," says Janet. "The difference for me now is that because of the PRIDE program I know how to lose weight, and I know I can be successful at it."

About Urinary Incontinence

UI is caused when the bladder muscles squeeze too often—or when you don't want them to. It can also occur if muscles around the bladder opening are not strong enough to hold back urine. Women usually experience "stress" or "urge" UI, and sometimes both. Stress UI is the leakage of small amounts of urine during physical activity, such as coughing, sneezing, and exercising. Urge UI is the leakage of large amounts

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of urine at unexpected times, including during sleep. Janet says she has experienced both types, but fortunately never has had an incident while sleeping.

Understandably, UI is associated with diminished quality of life. But, the good news is that the symptoms of UI can often be treated, and thanks to the PRIDE study it is now confirmed that losing weight can now be included as one of the treatment approaches that should be considered for women who are overweight or obese.

As for Janet, she says she would "absolutely" recommend studies like PRIDE to others. She says that the PRIDE staff made her feel accountable to herself, as well as to them. "And I'll have the knowledge they gave me forever," she says.

For additional information on UI—

For Women:
http://kidney.niddk.nih.gov/kudiseases/pubs/uiwomen/index.htm

For Men:
http://kidney.niddk.nih.gov/kudiseases/pubs/uimen/

For Children:
http://kidney.niddk.nih.gov/kudiseases/pubs/uichildren/